

Evaluation of juvenile hormone analogues as rodent feed-through insecticides for control of immature phlebotomine sandflies

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Abstract. The juvenile hormone analogues methoprene and pyriproxyfen were evaluated as rodent feed-through insecticides for control of immature stages of the sandfly *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae). The development and survival of *P. papatasi* second-instar larvae fed faeces from Syrian hamsters, *Mesocricetus auratus*, that had been fed a diet containing methoprene (0, 9.788, 97.88 or 978.8 p.p.m.) or pyriproxyfen (0, 9.82, 98.2 or 982 p.p.m.) were evaluated. The faeces of methoprene-treated hamsters greatly reduced the percentage of larvae that pupated at all concentrations tested and prevented adult emergence at all but the lowest concentration (9.788 p.p.m.). Pyriproxyfen prevented both pupation and adult emergence at all concentrations tested. The results of this study suggest that a control strategy using rodent baits containing juvenile hormone analogues to control phlebotomine sandflies that live in rodent burrows and feed on rodent faeces may be possible. As rodent reservoirs and vectors of *Leishmania major* live in close association in many parts of the Middle East, control of the transmission of the agent of zoonotic cutaneous leishmaniasis may also be possible.

Key words. *Phlebotomus papatasi*, juvenile hormone analogues, methoprene, pyriproxyfen, sandfly control.

Introduction

Phlebotomine sandflies are significant biting pests of humans and are the vectors of several human pathogens including *Bartonella bacilliformis*, sandfly fever Sicilian virus and sandfly fever Naples virus. Most importantly, sandflies are the vectors of the protozoan parasites that cause leishmaniasis. Worldwide, two million new cases of leishmaniasis are believed to occur annually and as many as 12 million people may currently be infected (World Health Organization, 2008).

The sandfly species *Phlebotomus papatasi* Scopoli occurs in Mediterranean littoral countries and throughout southwestern and central Asia. In arid areas within its distribution, *P. papatasi* is the vector of *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis (ZCL). The reservoir

hosts of *L. major* are various species of locally abundant burrowing rodents. Sandflies aggregate within rodent burrows, which provide the microclimatic conditions they require for survival (darkness, high relative humidity, protection from extreme temperatures). Adult sandflies live in close proximity to sources of blood (from the rodents living within the burrows) and sugar (from plants that grow near the burrow entrances), and sandfly larvae develop within the organic matter inside the burrows.

The close association between sandflies and rodent burrows has been demonstrated in many different sandfly–rodent associations in Old World ZCL foci. However, targeting burrows with insecticides has not been effective at controlling sandfly populations because insecticide applications in and around rodent burrows do not reach deep within the burrows

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where adult and immature sandflies are located (Seyedi-Rashti & Nadim, 1973; Karapet'ian *et al.*, 1983). Because leishmaniasis is an emerging disease that disproportionately affects human populations in developing countries, the development of new and efficacious methods for the control of vectors of ZCL is needed (Saravia, 2004).

The primary habitat for immature *P. papatasi* in ZCL foci is considered to be organic debris in rodent burrows and sandfly larvae have been observed feeding on the faeces of rodents (World Health Organization, 1968). Therefore, the use of rodent feed-through insecticides may be a potential method to control sandfly larvae. Proof of concept for rodent feed-through control of larvae of *P. papatasi* has been established in laboratory studies using two benzoylurea chitin synthesis inhibitors (diflubenzuron and novaluron) and a macrocyclic lactone (ivermectin) (Mascari *et al.*, 2007a, 2007b, 2008). The objective of this study was to evaluate the juvenile hormone analogues methoprene and pyriproxyfen as rodent feed-through insecticides to control sandfly larvae. The development and survival of *P. papatasi* larvae fed faeces of Syrian hamsters, *Mesocricetus auratus*, that had been fed a diet containing methoprene or pyriproxyfen were measured.

Materials and methods

Sandflies

The sandflies used in these studies were sourced from a laboratory colony of a Turkish strain of *P. papatasi* established at Louisiana State University (Mascari *et al.*, 2007b). The sandfly larvae in the colony were reared using a larval diet composed of a composted and dried 1 : 1 mixture of rabbit faeces and rabbit chow (Young *et al.*, 1981). Adult sandflies were provided with 20% sucrose solution *ad libitum* and obtained bloodmeals from Syrian hamsters. The colony was maintained in environmental chambers at 28 °C, 90% relative humidity (RH), under an LD 14 : 10 h photoperiod.

Syrian hamsters

A total of 24 Syrian hamsters were housed individually in micro-isolator cages. The maintenance of the hamsters and all experimental procedures followed Animal Care and Use Protocol No. 05-074, which was approved by the Institutional Animal Care and Use Committee at Louisiana State University, Baton Rouge, LA, U.S.A. Research involving the hamsters was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adhered to principles set out by the National Research Council (1996).

Feed-through

Hamster diets were prepared by adding pyriproxyfen [98.2% active ingredient (a.i.); Valent U.S.A. Corp., Walnut Creek, CA, U.S.A.] and methoprene (97.88% a.i.; Central Life Sciences, Walnut Creek, CA, U.S.A.) to a meal-form laboratory rodent

diet (5001 Rodent Diet, LabDiet®; PMI Nutrition International, Brentwood, MO, U.S.A.). Pyriproxyfen was added directly to hamster food to achieve three concentrations (9.82, 98.2 or 982 p.p.m.) and the diets were thoroughly mixed. An untreated control diet was also prepared. The technical methoprene was in liquid form and was diluted in pure soybean oil before being added to hamster food. Diluted methoprene was added to hamster food at a rate of 100 g to 900 g of powdered hamster food, yielding hamster food containing three concentrations of methoprene: 9.788 p.p.m., 97.88 p.p.m. and 978.8 p.p.m. An additional control diet was prepared by adding soybean oil at a rate of 100 g to 900 g of hamster food.

Three hamsters were randomly assigned to each of the eight diet groups (three concentrations of pyriproxyfen, three concentrations of methoprene, a soybean oil control diet group, and an untreated control diet group). The hamsters were provided with 25 g of their respective diets each day for 9 days. Any uneaten portion of the food was collected the following day and the daily food intake for each hamster was calculated. The daily dosages of pyriproxyfen and methoprene ingested by the hamsters were calculated in mg/kg of bodyweight (the bodyweight of the hamsters was measured on the day before the experiment). The faeces voided by each hamster were collected daily for 9 days. All faeces were dried at room temperature for 7 days and were then stored at -80 °C until used.

The daily food intake of hamsters in each of the eight diet groups was compared using repeated-measures analysis of variance (ANOVA), performed with the general linear model (GLM) procedure in SAS (SAS Institute, 2001). Tukey's multiple comparison procedure was used to separate significantly different means. The daily dosages of pyriproxyfen or methoprene for individual hamsters were compared within hamster diet groups using the same method of statistical analysis.

Larval bioassay

Faeces voided by hamsters after 9 days of feeding on their respective diets were used as diets for sandfly larvae. The faeces were pooled by hamster diet group and manually crushed using a sterilized glass mortar and pestle.

Larval bioassays were conducted according to the methods described by Mascari *et al.* (2007a). A 0.4-g portion of faeces was transferred to the plaster surface of each bioassay vial. Ten second-instar (13 ± 1 -day-old) larvae were transferred to each bioassay vial and held in an environmental chamber at 28 °C, 90% RH, under an LD 14 : 10 h photoperiod. Five bioassay vials were used for each of the eight larval diet groups.

The larvae were observed under magnification daily. Mortality, which was defined as the lack of response to prodding with a blunt probe after 15 s, was recorded, and the sandflies were observed for abnormal behavioural and morphological characteristics. Evidence of feeding, which was defined by the presence of frass in the vials and dark material in the guts of larvae, was also monitored.

The percentage survival of sandfly larvae and the age of the larvae at death in each larval diet group (faeces of hamsters maintained on three concentrations of pyriproxyfen, three concentrations of methoprene, a soybean oil control diet, or an

untreated control diet) were compared with repeated-measures ANOVA performed with the GLM procedure (SAS Institute, 2001). Tukey's multiple comparison procedure was used to separate significantly different means.

Results

Feed-through

The mean bodyweight of the 24 hamsters in this study was 132.6 ± 6.4 g; the bodyweights of hamsters assigned to different diet groups did not differ significantly ($F = 0.03$, d.f. = 7, $P > 1.0000$). The mean daily food intake of the 24 hamsters in this study was 9.6 ± 1.8 g. There were significant differences in mean daily food intake between hamsters fed diets with soybean oil (0, 9.788, 97.88 and 978.8 p.p.m. methoprene) and without soybean oil (0, 9.82, 98.2, 982 p.p.m. pyriproxyfen) ($F = 17.64$, d.f. = 7, $P < 0.0001$). The mean daily food intake of hamsters fed diets containing soybean oil (10.7 ± 1.6 g) was 24.4% higher than that of hamsters fed a diet without soybean oil (8.6 ± 1.3 g). The amount of food eaten by hamsters in different diet groups containing soybean oil did not differ significantly ($F = 2.19$, d.f. = 3, $P = 0.0941$), and neither did the amount of food eaten by hamsters in different diet groups without soybean oil ($F = 0.30$, d.f. = 3, $P = 0.8242$).

The mean daily dosages of methoprene for hamsters were 0.8 ± 0.1 mg, 7.8 ± 1.3 mg and 80.5 ± 12.1 mg per kg bodyweight for hamsters fed diets containing 9.788 p.p.m., 97.88 p.p.m. or 978.8 p.p.m. methoprene, respectively. The mean daily dosages of pyriproxyfen for hamsters were 0.6 ± 0.1 mg, 6.5 ± 1.1 mg and 62.6 ± 11.3 mg per kg bodyweight for hamsters fed diets containing none, 9.82 p.p.m., 98.2 p.p.m. and 982 p.p.m. pyriproxyfen, respectively.

Larval bioassay

The sandfly larvae in each larval diet group were observed feeding, and frass was found in every vial. The mean percentage of adult emergence did not differ significantly between sandfly larvae that fed on faeces of hamsters maintained on an untreated diet or faeces of hamsters maintained on a diet containing untreated soybean oil ($F = 1.20$, d.f. = 1, $P = 0.3052$) (Table 1). Control larvae (larvae that had fed on faeces of hamsters maintained on an untreated diet or a diet containing soybean oil) first pupated when the sandflies were 24 days old. Adult emergence was first observed in both control groups when the sandflies were 30 days old.

The rates of pupation for larvae that fed on faeces of hamsters maintained on three concentrations of methoprene (9.788, 97.88 or 978.8 p.p.m.) were significantly lower than pupation rates of larvae in the control groups ($F = 89.62$, d.f. = 3, $P < 0.0001$) (Table 1). None of the larvae that were fed faeces of hamsters maintained on diets containing 97.88 p.p.m. or 978.8 p.p.m. methoprene emerged as adults. Only $4.0 \pm 5.5\%$ of the larvae that fed on faeces of hamsters maintained on a diet containing 9.788 p.p.m. methoprene

Table 1. Percentage pupation and adult emergence of immature sandflies fed faeces of hamsters maintained on diets containing methoprene or pyriproxyfen, or control diets. Five replicates of 10 larvae per replicate were performed for each diet.

Hamster diet, p.p.m.	Pupation, %, mean \pm SE*	Adult emergence, %, mean \pm SE*	Age at death, days, mean \pm SE
Control			
Untreated†	94.0 ± 8.9^a	94.0 ± 8.9^a	N/A
Soybean oil‡	90.0 ± 10.0^a	88.0 ± 8.4^a	N/A
Pyriproxyfen			
9.82	0 ^b	0 ^b	34.8 ± 6.0^a
98.2	0 ^b	0 ^b	34.2 ± 5.6^a
982	0 ^b	0 ^b	30.6 ± 5.3^a
Methoprene			
9.788	10.0 ± 14.1^b	4.0 ± 5.5^b	34.0 ± 7.5^a
97.88	2.0 ± 4.5^b	0 ^b	36.4 ± 7.2^a
978.8	8.0 ± 8.4^b	0 ^b	32.7 ± 4.7^a

*Values within a column followed by the same letter do not differ significantly from one another ($P > 0.05$).

†Untreated hamster food.

‡Rodent food + soybean oil (9 : 1 w/w).

SE, standard error; N/A, not applicable.

emerged as adults, which was significantly lower than the percentage of adult emergence of sandflies fed faeces of control hamsters ($F = 352.80$, d.f. = 1, $P < 0.0001$) (Table 1). The age at death of sandflies that, as larvae, fed on faeces of methoprene-treated hamsters was >32 days (Table 1).

In the pyriproxyfen larval bioassay, 100% mortality was observed during the larval stage for sandflies fed faeces of hamsters maintained on diets containing 9.82 p.p.m., 98.2 p.p.m. or 982.0 p.p.m. pyriproxyfen. The mean age of the larvae at death was >30 days (Table 1).

The majority of the sandfly larvae that were fed faeces of hamsters offered diets containing methoprene or pyriproxyfen died as late as the fourth stage. The larvae in these groups developed at a normal rate (the same rate as control larvae). Like the control larvae, the larvae in the treatment groups eventually ceased feeding and cleared their guts as late as the fourth stage. However, rather than progressing to the pupal stage, most of the sandfly larvae in the treatment groups remained as late fourth instars for up to 19 days before eventually dying. Some of the larvae that were fed faeces of methoprene- or pyriproxyfen-treated hamsters did transform from fourth-instar larvae into pupa-form larvae before dying (Fig. 1). These larvae developed normally as second-, third- and fourth-instar larvae, but became an intermediate form between larva and pupa after they ceased feeding and cleared their guts. Pupa-form larvae survived for several days, but eventually died without becoming pupae.

Discussion

The food intake of the hamsters in this study was not affected by the methoprene or pyriproxyfen treatments at any of the concentrations tested, and both compounds have very low toxicity to rodents (acute oral LD₅₀ in rats is $>34\ 600$ mg

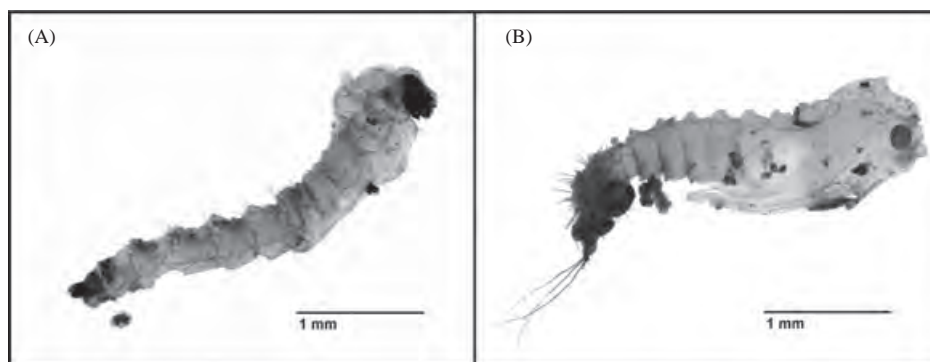


Fig. 1. (A) A pupa-form larva that, as a larva, had fed on faeces of hamsters maintained on a diet containing 9.82 p.p.m. pyriproxyfen, and (B) a normal pupa that, as a larva, had fed on faeces of untreated hamsters.

and >5000 mg per kg bodyweight for methoprene and pyriproxyfen, respectively; Food and Agriculture Organization/World Health Organization, 1985, 2000). This finding suggests that the treated diets were palatable to hamsters. Furthermore, significantly more food was consumed by hamsters when it contained soybean oil, independent of insecticide treatment. Bait preferences for the rodents involved in many of the sandfly–rodent associations found in Old World ZCL foci are known, and insecticide-treated baits could be developed for use in field trials in these scenarios. In southwest Asia, great gerbils (*Rhombomys opimus*) and jirds (*Meriones* spp.) are readily baited with oats. We are currently developing baits that target the fat sand rat (*Psammomys obesus*), which feeds largely on the leaves of plants in the family Chenopodiaceae. In sub-Saharan Africa, rodents of five genera known be reservoirs of *L. major* (*Mastomys*, *Taterillus*, *Aethomys*, *Tatera* and *Arvicanthis*) have been successfully captured in traps baited with corn flour (Githure *et al.*, 1986; Yaghoobi-Ershadi *et al.*, 2000, 2005).

The results of this study suggest that both methoprene and pyriproxyfen remained pharmacologically active after passing through the guts of hamsters, and that the compounds were present at sufficiently high concentrations to affect the development and survival of immature sandflies. As juvenile hormone analogues, both methoprene and pyriproxyfen were expected to have the same effect on the development of immature sandflies. The development of immature sandflies fed faeces of hamsters maintained on diets containing methoprene or pyriproxyfen was identical to that of control sandflies until they achieved fourth larval instar. Larvae that had been fed faeces of pyriproxyfen-treated hamsters remained as fourth-instar larvae or became pupa-form larvae, and all of these sandflies eventually died before pupation. However, pupation of larvae that fed on faeces of hamsters maintained on diets containing methoprene was observed for all concentrations, and adult emergence was seen at the lowest concentration. The finding that pyriproxyfen treatments fully prevented pupation and adult emergence at all concentrations tested, but similar concentrations of methoprene resulted in some pupation and adult emergence, is consistent with other studies that compared the effectiveness of methoprene and pyriproxyfen against other insects. The LC_{50} for methoprene was more than 20 times

higher than that for pyriproxyfen in an evaluation of the relative toxicity of methoprene and pyriproxyfen in topsoil against immature *Ctenocephalides felis* (Rajapakse *et al.*, 2002). Similarly, pyriproxyfen was found to be 21.5 times more toxic than methoprene to larvae of *Aedes albopictus* (Ali *et al.*, 1995). Against larvae of *Culex quinquefasciatus* and *A. albopictus*, methoprene provided significant but incomplete inhibition of adult emergence, even at the highest concentrations tested (Nayar *et al.*, 2002).

The results of this study add the juvenile hormone analogues methoprene and pyriproxyfen to the list of insecticides that can potentially be used as rodent feed-throughs for the control of phlebotomine sandflies in certain sandfly–rodent associations.

The identification of multiple insecticides that have been found to be effective as rodent feed-throughs against sandfly larvae in the laboratory increases the likelihood that a suitable compound will be found for use in field trials. However, future studies on the relative residual activity and environmental persistence of the compounds will be required before compounds can be recommended for field trials.

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References

- Ali, A., Nayar, J.K. & Xue, R.D. (1995) Comparative toxicity of selected larvicides and insect growth regulators to a Florida

- laboratory population of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, **11**, 72–76.
- Food and Agriculture Organization/World Health Organization (1985) Pesticide Residues in Food—1984 Evaluations. FAO/WHO, Geneva.
- Food and Agriculture Organization/World Health Organization (2000) Pesticide Residues in Food—1999 Evaluations. FAO/WHO, Geneva.
- Githure, J.I., Schnur, L.F., Le Blancq, S.M. & Hendricks, L.D. (1986) Characterization of Kenyan *Leishmania* spp. and identification of *Mastomys natalensis*, *Taterillus emini* and *Aethomys kaiser* as new hosts of *Leishmania major*. *Annals of Tropical Medicine and Parasitology*, **80**, 501–507.
- Karapet'ian, A.B., Dzhabarov, L.N., Mamigonova, R.I. & Sabatov, E.A. (1983) Use of D-20 aerosol insecticide smoke pots in controlling burrow sandflies. *Meditsinskaia Parazitologiya i Parazitarnye Bolezni*, **4**, 78–81.
- Mascari, T.M., Mitchell, M.A., Rowton, E.D. & Foil, L.D. (2007a) Laboratory evaluation of diflubenzuron as a feed-through for the control of immature sandflies (Diptera: Psychodidae). *Journal of Medical Entomology*, **44**, 171–174.
- Mascari, T.M., Mitchell, M.A., Rowton, E.D. & Foil, L.D. (2007b) Evaluation of novaluron as a feed-through insecticide for control of immature sandflies (Diptera: Psychodidae). *Journal of Medical Entomology*, **44**, 714–717.
- Mascari, T.M., Mitchell, M.A., Rowton, E.D. & Foil, L.D. (2008) Ivermectin as a rodent feed-through insecticide for control of immature sandflies (Diptera: Psychodidae). *Journal of the American Mosquito Control Association*, **24**, 323–326.
- National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, DC.
- Nayar, J.K., Ali, A. & Zaim, M. (2002) Effectiveness and residual activity comparison of granular formulations of insect growth regulators pyriproxyfen and s-methoprene against Florida mosquitoes in laboratory and outdoor conditions. *Journal of the American Mosquito Control Association*, **18**, 196–201.
- Rajapakse, C.N., Meola, R. & Readio, J. (2002) Comparative evaluation of juvenoids for control of cat fleas (Siphonaptera: Pulicidae) in topsoil. *Journal of Medical Entomology*, **39**, 889–894.
- Saravia, N.G. (2004) Can leishmaniasis still be considered a TDR category 1 disease? If moved, what is required? *Report of the Scientific Working Group Meeting on Leishmaniasis*, 2–4 February 2004, Geneva, 130–137.
- SAS Institute (2001) *SAS System for Windows*, Release 8.2. SAS Institute, Cary, NC.
- Seyedi-Rashti, M.A. & Nadim, A. (1973) Attempt to control zoonotic cutaneous leishmaniasis in the Isfahan area, Iran. *9th International Congress on Tropical Medicine and Malaria*, 14–21 October 1973, Athens, 135.
- World Health Organization (1968) WHO Inter-Regional Traveling Seminar on Leishmaniasis. WHO, Geneva.
- World Health Organization (2008) Leishmaniasis. <http://www.who.int/leishmaniasis/en/> [Accessed 10 June 2008].
- Yaghoobi-Ershadi, M.R., Akhavan, A.A., Zahraei-Ramazani, A.R., Javadian, E. & Motavalli-Emami, M. (2000) Field trials for the control of zoonotic cutaneous leishmaniasis in Badrood, Iran. *Annals of Saudi Medicine*, **20**, 386–389.
- Yaghoobi-Ershadi, M.R., Zahraei-Ramazani, A.R., Akhavan, A.A., Jalali-Zand, A.R., Abdoli, H. & Nadim, A. (2005) Rodent control operations against zoonotic cutaneous leishmaniasis in rural Iran. *Annals of Saudi Medicine*, **25**, 309–312.
- Young, D.G., Perkins, P.V. & Endris, R.G. (1981) A larval diet for rearing phlebotomine sandflies (Diptera: Psychodidae). *Journal of Medical Entomology*, **18**, 446.

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